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Development of microsporidia-infected *Muscidifurax raptor* (Hymenoptera: Pteromalidae) at different temperatures

Carl K. Boohene,[a] Christopher J. Geden,[b,*] and James J. Becnel[b]

[a] *Department of Entomology and Nematology, University of Florida Gainesville, FL, USA*
[b] *USDA-ARS-Center for Medical, Agricultural and Veterinary Entomology, P.O. Box 14565, Gainesville, FL 32604, USA*

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Abstract

Muscididfurax raptor, a pupal parasitoid of house flies and other filth flies, is commonly infected with the microsporidium *Nosema muscidifuracis*. To determine the effects of infection on developmental time, uninfected and infected adult *M. raptor* were allowed to parasitize pupae of the house fly (*Musca domestica*) for 24 h. Exposed pupae of the two groups (infected and uninfected) were held at 15, 20, 25, 30, 32, and 34 °C with 75–80% relative humidity. Development of infected *M. raptor* was significantly longer at all temperatures than that of uninfected parasitoids, resulting in approximately 7% extensions of developmental times. Uninfected females completed development in 14.6, 19.6, and 30.4 days at 30, 25, and 20 °C, respectively, compared with 15.8, 20.7, and 32.3 days for infected females at these temperatures. The differences in developmental times provided narrow windows for isolating large proportions of uninfected *M. raptor* females for disease management programs. This window was greatest at 20 °C; 61% of the uninfected females emerged by day 30, at which time only 10% of the infected females had emerged.
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Keywords: *Nosema muscidifuracis*; *Muscidifurax raptor*; Pteromalidae; Microsporidia; *Nosema*; Developmental time

1. Introduction

The family Pteromalidae contains several important parasitoids for the control of muscoid flies associated with dairy and poultry production (Axtell and Arrends, 1990; Geden et al., 1995). They can be easily and inexpensively mass-produced for inundative or inoculative releases to augment existing natural control (Morgan, 1981). Sustained releases of parasitoids in this family in integrated management programs have provided control of house flies and other muscoid flies (Geden et al., 1992a; Morgan et al., 1975). Several commercial insectaries are engaged in large-scale production of pteromalids, especially *Muscidifurax* spp. for the animal production industry. However, some control programs have met with mixed results, possibly due to infection of the parasitoids with microsporidia (Geden et al., 1992b; Rutz and Patterson, 1990).

The phylum Microsporidia contains a large and ubiquitous group of obligate intracellular parasites that produce spores containing a polar filament. They are found in all major animal phyla and are among the smallest eukaryotes known. Becnel and Geden (1994) have described the host parasite relationship for the parasitoid *Muscidifurax raptor* Girault and Sanders and the parasite *Nosema muscidifuracis* Becnel and Geden. The parasite infects the midgut epithelium, Malpighian tubules, ovaries and fat body of both larvae and adults (Becnel and Geden, 1994). The infection causes a chronic disease in adult parasitoids, resulting in reduced fecundity and longevity (Geden et al., 1995). The dipteran host of the parasitoid does not become infected. The transmission cycle in *M. raptor* involves both horizontal and vertical transmission. The disease is transmitted horizontally when healthy adult parasitoids feed on infected immatures within the host puparium and also when healthy parasitoid immatures cannibalize infected immatures within superparasitized hosts. Maternal transmission is nearly 100% efficient, and there is no paternal or venereal transmission (Geden et al., 1995).

* Corresponding author. Fax: +352-374-5922.
E-mail address: cgeden@gainesville.usda.ufl.edu (C.J. Geden).

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The detection of microsporidia in virtually every colony of *Muscidifurax* spp. in the world, including those maintained by commercial insectaries, has implications for biological control programs because infected parasitoids have reduced longevity, searching ability, and fecundity compared to uninfected parasitoids (Dry et al., 1999; Zchori-Fein et al., 1992). Prevalence of *N. muscidifuracis* varies between 1 and 11% in *M. raptor* populations on dairy farms where no parasitoids were released in contrast to 84% in farms where infected parasitoids were inadvertently released (Geden et al., 1995). It is therefore important for rigorous quality control measures to be implemented to ensure that infected parasitoids are not released in the field (Geden et al., 1995). Field surveys of pteromalids indicate that microsporidian infections of fly parasitoids are widely distributed and have been found in New York, Florida, Arkansas (Dry et al., 1999; Geden et al., 1995), South Korea and Brazil (CJG, unpublished data).

Strategies used to eliminate or reduce microsporidian infection in colonies include the Pasteur method, in which family lines are obtained from individual mated females that are examined for infection after oviposition. Heat and drug therapies have also proven to be effective in certain cases (Brooks et al., 1978; Cantwell and Shimanuki, 1969; Hsiao and Hsiao, 1973; Sheetz et al., 1997). Heat shock of infected *M. raptor* eggs within fly puparia was effective in eliminating *N. muscidifruacis* infections but the temperature required resulted in high host mortality (Geden et al., 1995). Because infection with microsporidia are known to result in delayed development times in some insects there is also the possibility that differential developmental times can be exploited to isolate uninfected individuals. The objective of this study was to determine whether infection of *M. raptor* with *N. muscidifuracis* would prolong the parasitoid's developmental time to such an extent that developmental windows could be identified that would allow the collection of large proportions of uninfected parasitoids as a disease management strategy.

2. Materials and methods

Muscidifurax raptor females ($N = 400$) infected with *N. muscidifuracis* were obtained from a colony maintained at the USDA-ARS Center for Medical, Agricultural and Veterinary Entomology (CMAVE), in Gainesville, Florida. The parasitoids were provided with 2400 1- to 2-day-old house fly (*Musca domestica* L.) pupae for oviposition for 24 h at 25 °C. Similarly, 2400 *M. domestica* pupae were exposed to 400 females from an uninfected colony for 24 h at 25 °C. After exposure to the parasitoids, each set of pupae was divided into six groups of 400 pupae. The pupae were then placed in small round paper cups (9 cm in diameter and 5 cm in depth) with a cover. One group from each set of cups containing pupae (i.e., 400 pupae from the infected group and 400 pupae from the uninfected group) was placed in a plastic container ($36 \times 25 \times 12$ cm) containing saturated NaCl solution to maintain constant humidity of 75–80% RH. The six groups were each placed inside environmental chambers set at 15, 20, 25, 30, 32, and 34 °C, respectively. Temperature and humidity conditions in the chambers were monitored with Stow-Away data loggers (Onset Computer Corporation, Pocasset, MA).

The containers were checked daily for fly emergence. Flies that emerged were discarded and uneclosed pupae were counted, placed individually in standard no. 2 gelatin capsules, and returned to the chambers for parasitoid emergence. The containers were monitored twice daily at 12-h intervals for parasitoid emergence until 7 days after emergence of the last parasitoid. Emerged parasitoids were counted and sexed during this period and developmental times were determined for each individual. They were assessed for infection status by placing each insect on a microscope slide with a drop of deionized water and a cover slide. The insect was then crushed and examined at 400× for the presence of spores. The entire experiment was replicated twice using different cohorts (parasitoids from different parents) of parasitoids.

Developmental time data were analyzed separately for males and females using the General Linear Models procedure of the Statistical Analysis System (SAS Institute, 1987), with temperature, infection status and temperature × infection status as the grouping variables. An a posterori means separation analysis was performed to examine the effect of infection on developmental time for each temperature. Emergence success of infected versus uninfected parasitoids was compared by conducting separate G-tests of independence for males and females at each temperature (Sokal and Rohlf, 1981). The effect of temperature on emergence success was evaluated by comparing parasitoid emergence at 25 °C with emergence at other temperatures using separate G-tests of independence for both sexes and infection status groups (Sokal and Rohlf, 1981).

3. Results

No uninfected parasitoids emerged when reared at 34 °C, and only one emerged at this temperature in the infected group (not presented in a table). At the remaining temperatures, the average number of hosts attacked by uninfected parasitoids was 380.0 ± 5.6 pupae out of the 400 that were provided; these pupae produced 287.0 ± 21 progeny ($n = 10$ sets of exposed host pupae). In contrast, infected parasitoids attacked 238.2 ± 37 pupae and produced 127.5 ± 37 progeny (Table 1). In-

Table 1
Emergence of uninfected *Muscidifurax raptor* and *Muscidifurax raptor* infected with *Nosema muscidifuracis* at different temperatures

Sex	Temperature (°C)	No. of parasitoids emerged			% Emergence (relative to 25 °C)			
		Uninfected	Infected	χ^2	Uninfected	χ^2	Infected	χ^2
Females	15	188	32	109.1**[a]	78.7	6.1*[2]	43.5	16.4**[b]
	20	205	58	75.5**	85.5	2.6ns	79.4	1.7ns
	25	239	73	78.6**	100.0	—	100.0	—
	30	214	57	83.7**	89.5	1.4ns	78.1	2.0ns
	32	233	49	112.5**	97.5	0.1ns	67.1	4.7*
Males	15	234	112	18.9**	69.9	18.0**	61.5	16.8**
	20	277	148	31.6**	82.7	5.5*	81.3	3.5ns
	25	335	182	34.9**	100.0	—	100.0	—
	30	346	171	42.1**	104.2	0.2ns	97.2	0.1ns
	32	311	141	51.4**	92.8	0.9ns	78.2	5.2*

[a] G-test of independence (df = 1) comparing emergence of infected versus uninfected parasitoids at each temperature. **, $P < 0.01$; *, $P < 0.05$, ns, $P > 0.05$.

[b] G-test of independence (df = 1) comparing emergence of infected or uninfected parasitoids relative to emergence at 25 °C. **, $P < 0.01$; *, $P < 0.05$, ns, $P > 0.05$.

fected parasitoids produced 75.3% fewer females and 49.9% fewer males than uninfected parasitoids. The effect of infection on emergence success was also modulated by temperature. Both infected and uninfected parasitoids had significantly lower emergence success at 15 °C compared with 25 °C. In contrast, exposure to 32 °C resulted in significantly lower relative emergence of infected parasitoids of both sexes but not of uninfected parasitoids.

Developmental times of infected parasitoids were significantly longer than those of uninfected parasitoids for both males and females (Tables 2 and 3). This effect was significant for females at all temperatures except 15 °C and resulted in extending the developmental time of infected parasitoids by 6.3, 4.7, 8.3, and 11% at 20, 25, 30, and 32 °C, respectively. For males, infection resulted in significant lengthening of developmental times at all temperatures. Infected males required 6.3, 6.1, 4.8, 7.5 and 7.4% more time to develop than uninfected females at 15, 20, 25, 30, and 32 °C, respectively.

Table 2
Developmental times (egg to adult) of male and female *Muscidifurax raptor* and *Muscidifurax raptor* infected with *Nosema muscidifuracis* at different temperatures

Sex	Temperature (°C)	Mean (SE) dev time (days)	
		Uninfected	Infected
Females	15	67.1 (0.3)a[a]	67.9 (0.9)a
	20	30.4 (0.1)a	32.3 (0.2)b
	25	19.6 (0.1)a	20.7 (0.2)b
	30	14.6 (0.1)a	15.8 (0.1)b
	32	14.2 (0.1)a	15.8 (0.2)b
Males	15	61.4 (0.3)a	65.2 (0.6)b
	20	27.8 (0.1)a	29.5 (0.1)b
	25	17.9 (0.1)a	18.7 (0.1)b
	30	13.4 (0.4)a	14.4 (0.1)b
	32	13.2 (0.1)a	14.1 (0.1)b

[a] Means followed by the same letter within a row are not significantly different at $P = 0.05$ based on the Ryan–Einot–Gabriel–Welsch multiple range test.

The distribution of developmental times for females is shown in Fig. 1, which expresses emergence in each interval as a proportion of the total in order to facilitate comparison of emergence patterns of infected versus uninfected parasitoids (more healthy parasitoids emerged than infected ones). Emergence at 15 °C occurred over 25 days for both infected and uninfected parasitoids, and there were no clear time intervals when only infected or uninfected individuals emerged (Fig. 1A). At 20 °C emergence occurred over 10 days; however, there was a clear separation in emergence patterns between uninfected and infected individuals. The first uninfected parasitoids emerged 12 h before the first infected ones, and 61% of the uninfected parasitoids had emerged by day 30 compared with 10% of the infected individuals (Fig. 1B).

At 25 °C, 22% of the uninfected parasitoids had emerged in the 36 h before emergence of the first infected individual, and 37% of the uninfected females had emerged by day 19 compared with 8% of the infected females (Fig. 1C). At 30 °C, 16% of the uninfected parasitoids had emerged in the 24 h before emergence of the first infected individual, and 40% of the uninfected parasitoids had emerged by day 14 compared with 4% of the infected females (Fig. 1D). Emergence at 32 °C was compressed into a short time interval, with most parasitoids emerging over a 6-day period between days 12 and 17 (Fig. 1E). Almost 60% of the uninfected parasitoids had emerged by day 14 compared with 12% of the infected parasitoids, but the time of emergence of the first individuals of both infection classes was similar.

Distribution patterns of developmental times for males, in general, were similar to the females, with the uninfected males emerging first at all temperatures (Fig. 2). At 15 and 20 °C (Fig. 2A and B, respectively), the

Table 3
Analysis of variance for the effects of temperature and infection with *Nosema muscidifuracis* on developmental times of male and female *Muscidifurax raptor*

Sex	ANOVA effect	df	*F*	Significance level
Females	Temp.	4, 1347	320.93	<0.0001
	Infection	1, 1347	28037.12	<0.0001
	Temp * Inf.	4. 1347	1.42	0.2260
Males	Temp.	4, 2252	35364.91	<0.0001
	Infection	1, 2252	317.83	<0.0001
	Temp * Inf.	4, 2252	32.78	<0.0001

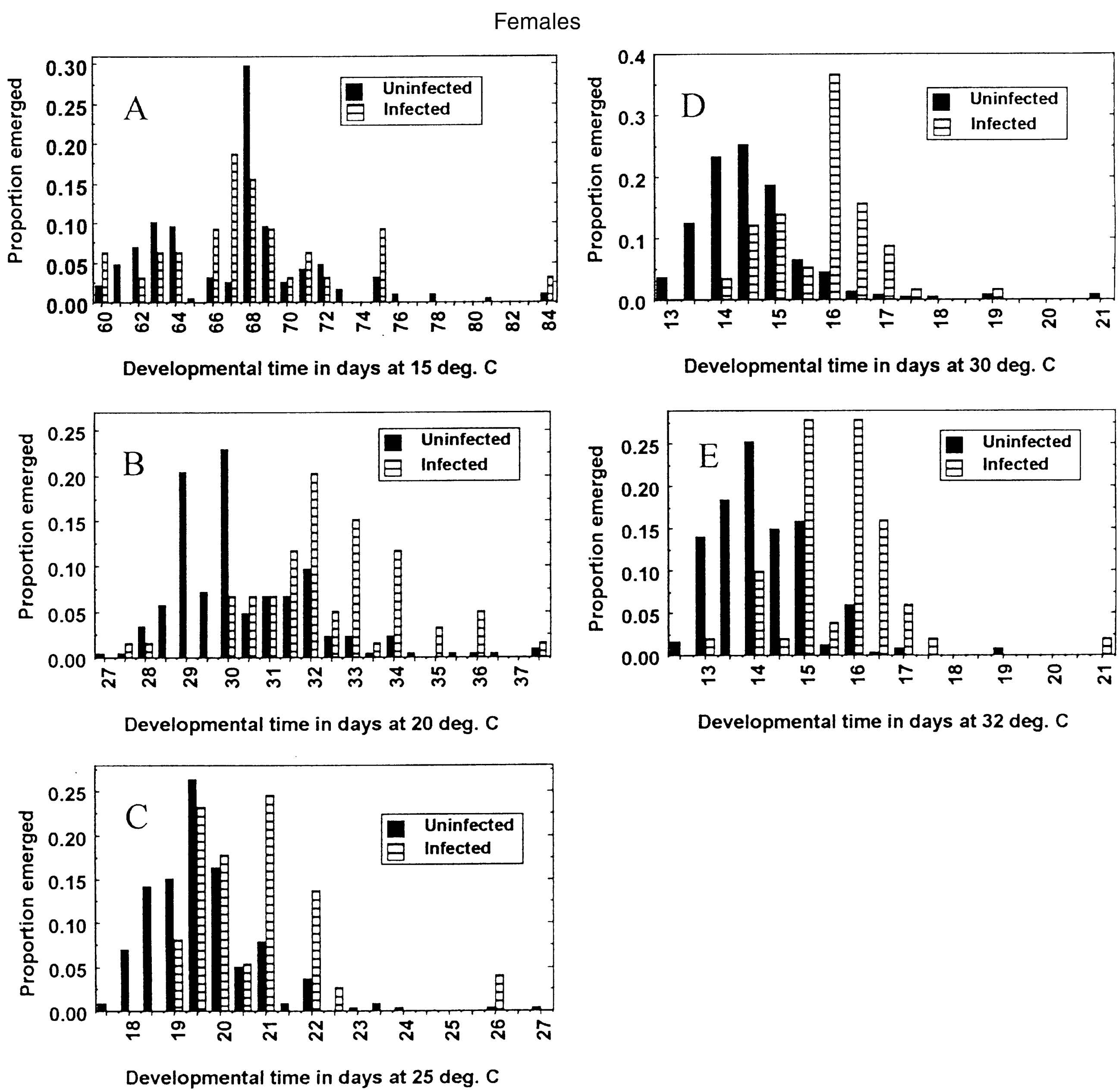

Fig. 1. Distribution of developmental times of uninfected female *Muscidifurax raptor* and of females infected with *Nosema muscidifuracis* at five temperatures.

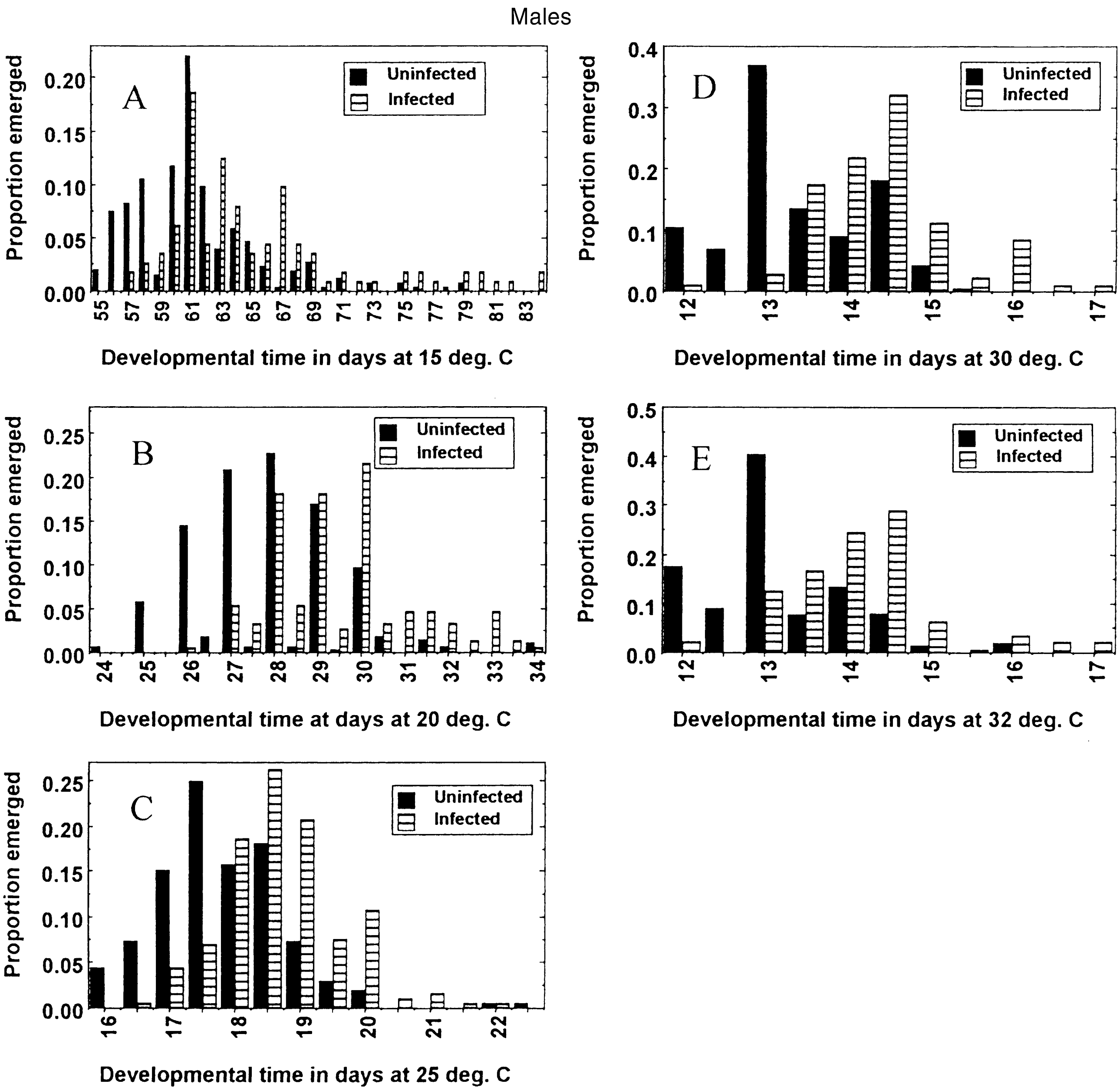

Fig. 2. Distribution of developmental times of uninfected male *Muscidifurax raptor* and of males infected with *Nosema muscidifuracis* at five temperatures.

first 2 days of emergence yielded only uninfected parasitoids. At 25, 30, and 32 °C, the majority of the uninfected males emerged within the first 2 days.

4. Discussion

Nosema muscidifuracis causes a chronic and debilitating disease in *M. raptor* which reduces the fitness of the parasitoid with respect to reproductive potential, longevity, and ability to locate and parasitize hosts (Geden et al., 1995; Zchori-Fein et al., 1992). Our data on relative attack rates and progeny production by infected and uninfected *M. raptor* confirm previous observations that infected individuals have lower fecundity than healthy parasitoids and that poor colony performance is not merely due to differential mortality of adults. Various developmental stages of the parasite are found in the ovaries, fat bodies, Malpighian tubules and midgut epithelium (Becnel and Geden, 1994). The infection of the ovaries and fat bodies of the adult females may be responsible for the reduced fecundity and longevity because the fat body is the storage site for nutrients in insects, and the females are dependent for the protein reserves needed for egg maturation (Gaugler and Brooks, 1975; Mitchell and Cali, 1994).

There are no gross differences in appearance between infected and uninfected *M. raptor*, although Geden et al. (1992b) did observe that infected individuals were somewhat smaller-bodied than healthy parasitoids. In practice, infected colonies usually go unnoticed until there is a marked deterioration in colony production parameters (Geden et al., 1992b). Microsporidian infections may be common in colonies of other beneficial arthropods. Such infections have recently been found in several species of *Spalangia*, the encyrtid parasitoid *Tachinaephagus zealandicus* (Ashmead) (unpublished data) and in colonies of predacious mites, where they also impact the overall fitness and effectiveness of these biological control agents (Bjornson and Keddie, 1999; Hoy, personal communication).

In our study, infected parasitoids produced progeny with a higher proportion of males than did uninfected parasitoids. The reason for this is unclear but may be due to poor mating success of infected males or to higher mortality among infected females in the immature stages. Because infected females contain more spores than males (Zchori-Fein et al., 1992), infection may have resulted in differential mortality of females compared with males.

Our developmental times for uninfected *M. raptor* are in agreement with those obtained by Geden (1997) at 15 20, 25, 30, and 32 °C. We observed no emergence at 34 °C, and Geden (1997) reported very low emergence at 35 °C, indicating that these temperatures represent the upper limit of tolerance for *M. raptor*. The duration of parasitoid emergence was longer at the lower temperatures, spanning about 25 days at 15 °C, about 10 days at 20 and 25 °C, and about 8 days at 30, and 32 °C. Approximately 90% of the parasitoids emerged within 4 days at 25, 30, and 32 °C.

Infected parasitoids took longer to develop than uninfected parasitoids under nearly all test conditions (Table 2). Many authors have observed that insects infected with microsporidia have prolonged developmental times (Becnel and Undeen, 1992; Gaugler and Brooks, 1975). Gaugler and Brooks (1975) found that sublethal infection of the corn earworm (*Helicoverpa zea*) with *N. heliothidis* retards larval development and results in deformed pupae. In contrast, Brooks and Cranford (1972) found no obvious effects of *Nosema campoletidis* in infected *Heliothis* spp., although they observed mortality effects when infected with *N. heliothidis.*

The reason for delayed development in infected *M. raptor* is not known. Delayed development of the immature stages of insects infected with microsporidia may be due to depletion of nutritional reserves, consumption of less food material, and a reduced ability to assimilate food efficiently (Johnson and Pavilikova, 1986; Thomson, 1958; Veber and Jasic, 1961). Based on observations made during other experiments with infected *M. raptor*, there does not appear to be a significant lengthening of the egg stage in infected colonies (CJG, unpublished data). Additional research involving dissections of parasitized hosts at various intervals would be required to determine which immature stages are most affected by infection.

Our data demonstrate that there are substantial differences in development times of infected versus uninfected *M. raptor* and that these differences are large enough to exploit in efforts to manage the disease in those instances where colony infection rates are less than 100%. Data in Fig. 1 indicate that large proportions of healthy females can be collected by retaining only those females that emerge within 24 h of emergence of the first female at 25 °C or within 48 h of emergence of the first female at 20 °C. Some of these uninfected females may have mated with infected males but the absence of paternal or venereal transmission of this pathogen makes this unimportant from the standpoint of colony health (Geden et al., 1995). This method can be used as part of an overall strategy incorporating sanitation, heat shock therapy, drug therapy, and monitoring to detect and manage microsporidioses in colonies of beneficial insects.

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